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Gender and age blood parameters references in African catfish (*Clarias gariepinus*) cultured in earthen ponds during dry season in Ibadan metropolis, Nigeria

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Abstract

Blood analysis is important in fish health investigation and may be influenced by intrinsic and extrinsic factors. However difficulties exist in the use of blood analysis in the investigation of fish health due the paucity of species specific, gender and age based reliable references. The current study therefore aims to determine some blood parameters in order to provide reliable references for dry season of the normal condition values of C. gariepinus a widely cultured fish in Nigeria. This study involved random sampling of 900 C. gariepinus aged 4, 5 and 6 months from earthen pond for three years. Data obtained for blood parameters using standard methods were analyzed using descriptive statistic, students'-test, one-way ANOVA level of significant set at p≤0.05. Results showed the females had significantly (p≤0.001) higher values than the males for each age bracket and the values increased significantly (p≤0.05) with increased in PCV, hemoglobin, RBC, WBC, total protein, potassium, sodium, Creatinine, ALT, AST and glucose The females and males values at 4, 5 and 6 months old were PCV (32.14±0.19% vs 26.72±1.12; 36.45±1.39% vs 32.33±1.46 %; 40.83±0.73% vs 35.01 ± 0.08%.), total protein (3.98±0.16g/l vs 3.49±0.18g/l; 4.45±0.10g/l vs 3.95±0.14g/l; 5.65 ± 0.11g/l vs 4.33 ± 0.04g/l.), Lymphocytes, neutrophils, monocytes and ESR values showed no stable trend in gender differences with increased age. In both the females and the males lymphocytes decreased significantly (p≤0.05) while neutrophils and monocytes increased significantly (p≤0.05) with increased age. ESR decreased significantly (P ≤ 0.05) in the males and non-significantly in the females with increase in age. It is hoped that these values obtained would serve as a reliable reference to enhance an appropriate intervention in the culture of C. gariepinus aged 4, 5 and 6 months in earthen pond which may be extrapolated for use in other culture systems.

Keywords: C. gariepinus, gender, blood parameters, earthen pond, culture.

Introduction

Fish is the cheapest source of animal protein consumed by the average Nigerian, accounting for about 40% of the total protein intake (Atanda, 2007). Inference from Ibe (1989) and Tewe (1997) documented figures presents a decline in protein of animal origin intake in Nigeria. Nigeria as the largest aquaculture producer in Africa, is rapidly expanding at 25–33% per year with a total estimated national demand of fish is as high as 2.66 million metric tons have a gap of over one million metric tons per annum Abba (2011). To bridge this gap which should be through aquaculture requires attention on the factors that influence fish performance which include nutrition, disease, environmental stress and pollutants as reported by Lebelo et al. (2001).

Since it has been widely demonstrated that cultured fish are more susceptible to disease agents than their wild counterparts due to the artificial conditions posed by intensive rearing (Irene et al., 2006) and that adverse environmental situations may acutely or chronically stress the health of fish, altering some of their biochemical parameters and suppressing their innate and adaptive immune responses (Giro'n-Pe'rez et al., 2007). The evaluation of blood cells, blood biochemistry and hormones could be useful for the diagnosis of fish disease and to monitor the physiological status of fish (Stoskopf, 1993) which was supported by the findings that blood analysis is crucial in many fields of fish farming, management and diseases investigation (Adedeji et al., 2009) and that that changes in the physiological state often reflect alteration of hematological and biochemical values Fazlolahzadeh et al. (2011). These hematological parameters in fish may be influenced by intrinsic factors (Joshi, 1982; Nespolo and Rosenmann, 2002) and by external factors (Mahajan and Dheer, 1979; Rois et al., 2002) may be responsible for the documentation that a large variation in hematological and biochemical parameters exists (Shalaby et al., 2006).

The knowledge of the normal range of blood parameters to compare with a deviation is necessary before it can be used as biomarkers (Luskova, 1990), also Adeyemo et al. (2009) in addition to documenting that one of the difficulties in assessing the state of health using hematological parameters of natural fish population is the paucity of reliable references of the normal condition data between genders. Therefore the main objective of this study is to determine some blood parameters based on gender and age in field condition for a reliable references to the normal condition values of *C. gariepinus,* a widely cultured fish in Nigeria owing to their high market price, fast growth rate and ability to withstand adverse pond conditions especially low oxygen content (Adewolu and Adeoti, 2010) during dry season in Ibadan.

Methodology

This involved the random sampling a total of 150 female and 150 male of apparently healthy catfish *C. gariepinus* using the criteria as suggested by Wedemeyer et al. (1977) aged 4, 5, and 6 months (50 each year) for three years from some private earthen fish pond farms during dry season. Sampling was done in the morning between 7:00 and 9:00am at an average ambient temperature of 26.3±0.4°C. All fish once cropped were gently wiped and properly restrained to cause minimal stress. The time interval for each fish sample out of pond water, restrain and blood collection was about 4 minutes so that the negative effects of stress due to handling on hematological and blood chemistry do not become significant. At the site of sampling, blood of each fish sampled was collected by caudal transection as described by James and William, (2001) using the lateral line as a landmark. A 21 gauge needle attached to 5ml syringes was used to collect blood from the severed (using a sterile razor blade) caudal vein. Approximately 3.0ml of blood was collected from each fish. About 2ml was immediately dispensed into a heparinzed (Adeyemo et al., 2009; Hattingh, 1995) commercial tube by Meus Srl Piove Disacco-Italy for hematology, while the remaining was dispensed into plain tube for serology. Appropriately labeled blood samples in a container containing ice cubes, were transported to the laboratory for the determination of blood parameters and stored at 0°C (Akiwande et al., 2004) in deep freezer prior analysis. The blood samples were analyzed within 3 hours of collection to ensure accurate laboratory results (Adeyemo et al., 2009).

Plasma was obtained after the determination of hematological parameters from the remaining blood in the heparinized tubes by spinning down at 1200 rpm for 1 minute for total protein, determination. Serum was obtain from the blood collected in plane non heparinized test-tubes was after clotting at room temperature for 1 hour and spine at 1200rpm for 5 minutes. The serum was harvested using sterile Pasteur pipette and stored at -20°C for biochemical parameters estimation.

- **Packed cell volume (PCV) determination:** 3/4 of microhaematocrit (heparinized) capillary tube was filled with blood from appropriately labled whole blood sample in heparinezed tube by capillary action. One end of the tube was sealed with plastercine. The tube was spined at 1,200rpm in microhaematocrit centrifigue for 5 minutes (Jain, 1986). The PCV was read using microhaematocrit reader and expressed as a percentage of the total blood volume.
- **Hemoglobin concentration determination**: The concentration of hemoglobin (Hb) was determined by cyanohaemoglobin method (Jain, 1986). Briefly 20µl blood was mixed with 4ml of modified drabkin's solution (potassium ferricyanide, 200mg, potassium cyanide, 50mg, potassium dihydrogen phosphate 140mg, volume made up to one litre with distilled water and pH adjusted to 7.0. This mixture was allowed to stand for 3 minutes before the hemoglobin concentration was read with Autospectrophotometer (spectrum lab 23A) at a wavelength of 540nm. The actual value of Hb concentration was extrapolated from a standard hemogblobin curve. The Drabkin's solution was kept in a dark cupboard after preparation for 36hr before using and returned immediately after use.
- **Determination of total red blood (RBC) Counts:** Total red blood cells and white blood cell counts were done manually (Adedeji et al., 2009; Zinkl, 1986) because nucleated RBC prevents accurate enumeration using automated analysis (Huffman et al., 1997). By the use of Neubauer hemocytometer according to Jain (1986) and the number of cells were expressed as 10^{12} Rbc per litre of blood and 10^{9} white blood cells per litre of blood respectively.
- Procedure: 1ml of RBC diluting fluid (Daciers fluid) was taken into test tube. 0.05µl of whole blood was added into the Daciers fluid (99ml of 3 percent aqueous solution of sodium citrate, 1ml of 40 percent formaldehyde) to keep and preserve the shape of the cells. This was gently mixed and allowed to stand and counted at X40 magnification under the microscope.
- **Determination of white blood cell (WBC) counts:** Procedure: 0.5ml of WBC diluting fluid (3 percent aqueous solution of acetic acid to which 1 percent gentian violet was added) was taken into a test tube. 25µl of whole blood was added into the fluid and gently mixed thoroughly and allowed to stand. It was counted at x40 magnification.
- **Differential white blood cell (WBC) Counts:** Differential WBC counts were manual as described by Adedeji, et al. (2009) and Zinkl (1986).
- **Procedure:** Freshly prepared Giemsa stain solution was used. Preparation: 66ml of glycerol was measured into conical flask, 1g of giemsa powder added into it. The mixture was allowed to boil in the water bath for 90mins -120min and mixed at interval for thorough boiling then 10% xylene was added. After it had attained the desired time allotted, it was removed and allowed to cool before methanol (Analar grade) 66ml was added into it. It was then mixed thoroughly, sieved and kept in a dark cupboard for a week before using. The stain was used at 10% with distilled water.

Thin blood smear was done using clean glass slide. Capillary tube was filled with whole blood. This was done by capillary action. A drop of blood was poured on the clean glass slide, and then a spreader was used to evenly spread the blood at

angle 45° to make a very good thin blood smear. This was allowed to dry under room temperature, then fixed with methanol (Analar grade) and allowed to dry; it was then immersed in giemsa stain solution for 45min. After staining, it was allowed to dry, then viewed under the microscope at x100 magnification, using Leica Galen III microscope to identify and count blood cells present. Thin blood smear stained with Giemsa as described by Jain (1986) and Kelly (1984) was used; 200 white blood cells were enumerated and differentiated.

- **Determination of erythrocyte sedimentation rate (ESR):** Filled blood capillary tube completely with whole blood, then sealed it with plasticin and allowed to stand for 1hr. With a measuring ruler, the levels of plasma and the total column was measured.

The calculation of ESR expressed as mm/hr was:

Plasma level (cm) x 50min

Total length of column (cm)

- **Estimation of total protein:** The estimation of total protein was done using Randox Kit® Stock solutions A and B were mixed to form the working solution. 3ml of distilled water was mixed with 3ml of Biuret (working solution) and was used as blank. 2.9ml of the working solution + 3mls of distilled water + 100µ of plasma sample formed sample solution; both the blank and sample solution were rocked gently and incubated at 37°C for 10minutes. After incubation they were read using autospectrolab at the wavelength 540nm.

Total Protein (g/l) was calculated as:

Optical density of sample X 21.05

Optical density of standard 1

- **Estimation of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST):** The estimation of ALT was done using Quimicaclinica applicada South Africa (QCA) Alanine Aminotransferase Kit®. 1ml distroater and 1 drop of sabbaste in the kit was mixed and incubated at 37°C for 5minutes using Gesell Scharft fur labortechnik water incubator to form solution A. 0.1ml of standard solution in the kit was mixed with solution A and also incubated at 37°C for 20minutes using Gesell Scharft fur labortechnik water incubator. These samples were read at 550 nm wavelength. ALT (iu/L) was calculated using the following formular:

Optical density of sample X 30

Optical density of standard 1

- **The estimation of AST:** The same process as in estimation of ALT but the estimation of AST was done using Quimicaclinica applicada South Africa (QCA) Aspartate Aminotransferase Kit®.
- **Estimation of glucose**: The estimation of total glucose was done using Randox Kit® 0.10ml of Haparinised blood was deproteinised with Urinyl acetate (ml). 0.02ml of deproteinised blood and 0.2ml of reagent 1 were mixed gently and incubated at 20°C for 25 minutes and read at set wavelength of 546nm.

Calculation = Optical Density of SampleX Standard Coefficient X 100ml = mg/dl.

Estimation of potassium and estimation of sodium

The Kit used for the estimation of potassium was Teco diagnostics California, USA, potassium Kit® 10µl of serum + 1ml of potassium reagent provided in the kit were mixed properly and allowed to stand for 3 minutes at room temperature. It was thereafter, read at set wavelength of 500nm.

Potassium = Optical density of sample of sample X Standard

Standard = 4mg/l.

The kit used for the estimation of sodium was Teco diagnostics California USA sodium Kit®. 50µl of serum sample was mixed vigorously with 1ml reagent for 3 minutes then centrifuged for 10 minutes. 50µl of the supernatant was added to 1ml acid reagent provided in the kit. 50µl of colour reagent provided was added and mixed thoroughly then read at set wavelength of 550nm and calculated as follows:

Sodium = Optical density of sample X Standard concentration.

Standard concentration given is 150mg/l.

- **Estimation of creatinine:** Randox creatinine Kit® was used. 4ml of plasma and 6ml distilled water and 4ml of sulfuric acid and 2ml of sodium tungstate solution were mixed thoroughly and allowed to stand for 3 minutes and centrifuged then to 5ml of supernatant was added 7.5ml of picrate solution then read at set wavelength of 540nm.

Creatinine calculation = Optical density of sample X Standard coefficient. Standard coefficient is 96mg/l

- **Data Analysis:** All Data collected were coded and analyzed statistically using the procedure as described on the statistical package SPSS 15.0 on a Computer. One-way analysis of variance (ANOVA) was used to test the effects of dependent variables. The Latin square analyses were calculated with the rules according to the methods of Snedecor and Cochran (1969). The analysis t- testing for differences between means of different clinical variable were performed. In addition, Duncan (1955) multiple range test (DMRT) was used to test for significance for each variable. The *post hoc* comparison of means was carried out using Duncan's multiple range tests (Frank and Althoen, 1990).Duncan's multiple range tests; significance was accepted at the 5% level.

Results

Table 1: Hematological Parameters Means ± SD reference values of *C. gariepinus* in earthen pond culture.

Parameters	4 months old		5 months old		6 months old	
	Females♀	Males ♂	Females♀	Males ♂	Females♀	Males ♂
	n = 150	n = 150	n = 150	n = 150	n = 150	n = 150
PCV (%)	32.14±0.19^{b}	26.72±1.12^{**a}	36.45±1.39^{d}	32.33±1.46^{**b}	40.83 ± 0.73^{e}	35.01 ± 0.08^{**c}
Hb (g/dl)	10.42±0.43^{b}	8.83±0.38^{**a}	12.26±0.65^{c}	10.72±0.74^{**c}	13.64±0.18^{f}	11.69±0.08^{**d}
RBCcellsx10^{12}/L	8.39±1.19^{b}	4.20±0.23^{**a}	11.19±1.19^{d}	8.29±1.64^{**c}	12.63±0.10^{e}	10.09±0.18^{**c}
WBCcellx10^{9}/L	11.70±0.94^{c}	8.68±0.47^{**a}	13.70±0.69^{e}	10.37±0.41^{**b}	15.11±0.67^{f}	12.03±0.06^{**d}
Lymphocyte %	66.63±3.51^{c}	67.05±7.63^{c}	63.71±6.02^{b}	66.71±6.02^{**c}	58.95 ± 0.30^{a}	59.53 ± 0.83^{a}
Neutrophils %	33.00±3.47^{ab}	32.43±7.04^{a}	33.01±2.70^{a}	35.97±5.79^{**b}	41.27 ± 0.51^{d}	38.93 ± 0.73^{**c}
Monocyte %	0.31±0.46^{a}	0.63±0.68^{**c}	0.21±0.41^{a}	0.31±0.47^{a}	0.44±0.72^{c}	1.59 ± 0.51^{**d}
ESR mm/hr	1.93±0.72^{bc}	3.14±1.32^{**d}	1.87+1.15^{bc}	2.02±1.01^{c}	1.79 ± 0.42^{b}	1.33 ± 0.47^{**a}

*** Highly significantly different at P ≤ 0.001 (Student t test)*

Means with the same letter are not significantly different according to DMRT at P ≤ 0.05.

Table 2: Reference range of hematological parameters with age of *C. gariepinus*

Parameters	Age (in months)					
	4		5		6	
	Females	Males	Females	Males	Females	Males
PCV (%)	30–33	24–29	34–39	29–35	40–43	35–36
Hb(g/dl)	9–11	8–10	11–13	9–12	13–14	11–12
RBC cellsx10^{12}/L	6–10	3–5	6–10	3–5	12–13	9–12
WBC cellx10^{9}/L	9–13	7–10	12–15	9–11	14–16	11–13
Platelets %	9–10	5–11	10–15	9–11	16–16	10–12
MCV(fl)	28–46	61–64	30–35	33–46	33–33	34–34
MCH(pg)	11–15	20 - 21	9–12	11–15	10–11	11–12
MCHC (g/dl)	33–33	33–33	33–33	33–33	33–33	33–33
Lymph %	61–72	58–76	60–72	56–72	58–61	56–62
Neut %	27–39	24–41	28–40	28 - 43	39–42	37–44
Monocyte %	0–1	0–2	0–1	0–1	0–2	0–2
ESR mm/hr	1–3	1–5	1–5	1–4	1–2	1–2

- **Packed cell volume (PCV)**: At 4 months old, the females had higher highly significant (p≤0.001) value of 32.14+0.19 % than the males with 26.72+1.12. At 5 months old, the females had a higher highly significant (p≤0.001) value of 36.45±1.39 % than the males with 32.33±1.46 % and at 6 months old, the females had a higher highly significant (p≤0.001) value of 40.83+0.73% than the males with 35.01 + 0.08%. PCV in both the females and males had significant (P ≤ 0.05) increase with age (Table 1).
- **Hemoglobin (Hb):** At 4 months old, the females had a higher highly significant (p≤0.001) value of 10.42±0.43 g/dl than the males with 8.83±0.38g/dl. At 5 months old, the females had a higher highly significant (p≤0.001) value of 12.26±0.65 g/dl than the males with 10.72±0.74g/dl and at 6 months old, the females had a higher highly significant (p≤0.001) value of 13.64±0.18 g/dl than the males with 11.69±0.08/dl. Using DMRT, as seen in table 1, hemoglobin in both the females and males had significant (P ≤ 0.05) increase with age (Table 1).
- **Red blood cells (RBC):** At 4months old, the females had a higher highly significant (p≤0.001) value of 8.39+1.19 cellX10^{12}/l than the males with 4.20±0.23cellX10^{12}/l. At 5 months old, the females had a higher highly significant (p≤0.001) value of 11.19±1.19 cells10^{12}/l than the males with 8.29±1.64cellsX10^{12}/l and at 6 months old, the females had a higher significant (p≤0.001) value of 12.63±0.10 cellsX10^{12}/l than the males with 10.09±0.18cellsX10^{12}/l. RBC in both the females and males had significant (P ≤ 0.05) increase with age (Table 1).
- **White blood cell (WBC):** At 4 months old the females had a higher highly significant (p≤0.001) value of 11.70±0.94 cellsX10^{9}0/l than the males with 8.68±0.47cellsX10^{9}/l. At 5 months old the females had a higher highly significant (p≤0.001) value of 13.70±0.69 cellsX10^{9}/l than the males with 10.37±0.41cellsX10^{9}/l; at 6 months old the females had a higher significant (p≤0.001) value of 15.11±0.67 cellsX10^{9}/l than the males with 12.03±0.06cellsX10^{9}/l. WBC in both the females and males had significant (P ≤ 0.05) increase with age (Table 1).
- **Lymphocytes (lymph):** At 4 months old the females had a lower non-significant value of 66.63±3.51 % than the males with 67.05±7.63 %. At 5 months the females had a lower highly significant (p≤0.001) value of 63.71±6.02 % than the males with 66.71±6.02%. At 6 months the females had a lower non-significant value of 58.95 ± 0.30%

than the males with 59.53 ± 0.83 %. Using DMRT as seen in table 1, lymphocytes in the females at 4 and 5months values difference was non-significant but a higher significant (P≤0.05) value that at 6months. Lymphocyte in the males at 4months had a higher significant (P ≤ 0.05) value than at 5 and 6months (Table 1).

- **Neutrophils:** At 4 months old the females had a higher non- significant value of 33.00+3.47 % than the males with 32.43±7.04 %. At 5 months old the females had a lower highly significant (p≤0.001) value of 33.01±2.70 % than the males with 35.97±5.79%. At 6 months old the females had a higher highly significant (p≤0.001) value of 41.27 ± 0.51 % than the males with 38.93 + 0.73%. Neutrophils at 4, 5 and 6 months, the females had a significant increase at 6months old while the male had significant increase with increase age (Table 1).
- **Monocytes:** At 4 months old the females had a lower highly significant (p≤0.001) value of 0.31±0.46 % than the males with 0.63±0.68%. At 5 months old the females had a lower non-significant value of 0.21±0.41 % than the males with 0.31±0.47 %. At 6 months old the females had a lower highly significant (p≤0.001) value of 0.44±0.72 % than the males with 1.59 ± 0.51%. Monocytes in the females at 4 and 5months difference was non-significant but both had a lower significant (P ≤ 0.05) value than at 6months. In the males, at 4months had a higher significant (P ≤ 0.05) value than at 5 months, which was a lower significant (P ≤ 0.05) value than at 6months (Table 1).
- **Erythrocyte sedimentation rate (ESR):** At 4 months old the females had a lower highly significant (p≤0.001) value of 1.93±0.72 mm/hr than the males with 3.14±1.32mm/hr; at 5 months old the females had a lower non-significant value of 1.87±1.15 mm/hr than the males with 2.02±1.01 mm/hr; at 6 months old, the females had a higher highly significant (p≤0.001) value of 1.79 ± 0.42 mm/hr than the males with 1.33 ± 0.47mm/hr. ESR value differences in the females at 4, 5 and 6 months was non-significant. In the males there was a significant (P ≤ 0.05) decrease with increase in age (Table 1).

Table 3: Blood chemistry parameters Means ± SD reference values of young *C. gariepinus* in earthen pond culture.

Parameters	4 months old		5 months old		6 months old	
	Females♀	Males ♂	Females♀	Males ♂	Females♀	Males ♂
	n =150	n =150	n =150	n =150	n =150	n =150
Total protein (g/l)	3.98±0.16^{c}	3.49±0.18^{**a}	4.45±0.10^{e}	3.95±0.14^{**b}	5.65 ± 0.11^{f}	4.33 ± 0.04^{**d}
K^{+}(meg/l)	21.57±2.06^{c}	13.73±2.08^{**a}	34.05±5.06^{d}	18.77±2.68^{**b}	55.00 ±1.00^{f}	38.25 ± 0.44^{**e}
Na^{+}(meg/l)	34.31±2.17^{c}	25.01±2.15^{**a}	60.32±14.98^{c}	32.13±4.39^{**b}	96.74 ± 1.95^{f}	71.51 ± 1.67^{**e}
Creatinine mg/dl	1.09±0.00^{b}	1.03±0.04^{**a}	1.15±0.10^{d}	1.11±0.07^{**c}	2.45 ± 0.03^{f}	1.22 ± 0.00^{**e}
ALT (Int Units/l)	30.65±3.94^{c}	22.49±1.81^{**a}	34.75±1.59^{c}	28.01±4.00^{**b}	54.74 ± 2.36^{e}	34.25 ± 1.31^{**d}
AST (Int Units/l)	47.00±6.23^{b}	35.11±2.49^{**a}	52.77±2.60^{e}	47.91±4.84^{**c}	96.24 ± 1.80^{f}	49.00 ± 1.59^{**e}
Glucose(mg/dl)	51.23±2.30^{c}	44.21±2.05^{**a}	67.60±3.67^{e}	54.64±5.13^{**c}	83.00 ± 0.70^{f}	60.51 ± 0.87^{**b}

*** Highly significantly different at P≤0.001 (Student t- test)*
Means with the same letter are not significantly different according to DMRT at p≤0.05.

Table 4: Reference range of blood chemistry parameters with age of *C. gariepinus*.

Parameters	Age (Months)					
	4		5		6	
	Females	Males	Females	Males	Females	Males
Total protein g/l	3.70–4.20	3.00–3.70	4.20–4.60	3.60–4.20	5.50–5.80	4.30-4.40
K++(meg/l)	17.00–24.00	9.00–16.00	28.00–42.00	15.00–25.00	54.00-56.00	38.00-39.00
Na++(meg/l)	31.00–37.00	31.00–37.00	44.00–82.00	27.00–40.00	94.00-99.00	70.00-74.00
Creatinine mg/dl	1.00–1.11	1.00–1.10	1.00–1.24	1.00–1.21	2.42–2.50	1.21-1.22
ALT (Int Units/l)	26.00–36.00	20.00–25.00	33.00–37.00	20.00–32.00	52.00 - 58.00	33.00-36.00
AST (Int Units/l)	40.00–56.00	30.00–38.00	50.00–58.00	40.00-54.00	94.00-98.00	47.00-51.00
Glucose(mg/dl)	48.00-55.00	38.00–46.00	62.00–73.00	46.00–62.00	82.00-84.00	60.00-62.00

- **Total Protein:** At 4 months old the females had a higher highly significant (p≤0.001) value of 3.98+0.16g/l than the males with 3.49±0.18g/l. At 5 months old the females had a higher highly significant (p≤0.001) value of 4.45±0.10g/l than the males with 3.95±0.14g/l. At 6 month old the females had a higher highly significant (p≤0.001) value of 5.65 ± 0.11g/l than the males with 4.33 ± 0.04g/l. Total protein increased with increased age in both the female and male (Table 3).
- **Potassium (K^{+}):** At 4 months old, the females had a higher highly significant (p<0.001) value of 21.57±2.06meg/l than the males with 13.73±2.08meg/l. At 5 months old the females had a higher highly significant (p≤0.001) value of 34.05±5.06meg/l than the males with 18.77±2.68meg/l. At 6 months old the females had a higher highly significant (p≤0.001) value of 55.00 ±1.00 meg/l than the males with 38.25 + 0.44meg/l. Potassium increased with increased age in both females and males (Table 3).
- **Sodium (Na^{+}):** At 4 months old, the females had a higher highly significant (p≤0.001) value of 34.31±2.17meg/l than the males with 25.01±2.15meg/l. At 5 months old, the females had a higher highly significant (p≤0.001) value

of 60.32±14.98meg/l than the males with 32.13±4.39meg/l. At 6 months old the females had a higher highly significant ($p \leq 0.001$) value of 96.74 + 1.95meg/l than the males with 71.51 ± 1.67meg/l. Sodium (Na^+) increased with increased age in both the female and male (Table 3).

- **Creatinine:** At 4 months old, the females had a higher highly significant ($p \leq 0.001$) value of 1.09±0.00mg/dl than the males with 1.03±0.04mg/dl. At 5 months old, the females had a higher highly significant ($p \leq 0.001$) value of 1.15+0.10mg/dl than the males with 1.11±0.07mg/dl. At 6 months old the females had a higher highly significant ($p \leq 0.001$) value of 2.45 ± 0.03mg/dl than the males with 1.22 ± 0.00mg/dl. Creatinine increased with increased age in both females and males (Table 3).
- **Alanine aminotransferase (ALT):** At 4 months old, the females had a higher highly significant ($p \leq 0.001$) value of 30.65±3.94IU/L than the males with 22.49+1.81IU/L. At 5 months old, the females had a higher highly significant ($p \leq 0.001$) value of 34.75±1.59IU/L than the males with 28.01±4.00IU/L. At 6 months old the females had a higher significant ($p \leq 0.001$) value of 54.74 ± 2.36IU/L than the males with 34.25 + 1.31IU/L. ALT increased with increased age in both females and males. (Table 5.2)
- **Aspartate aminotransferase (AST):** At 4 months old, the females had a higher highly significant ($p \leq 0.001$) value of 47.00±6.23IU/L than the males with 35.11±2.49IU/L. At 5 months old, the females had a higher highly significant ($p \leq 0.001$) value of 52.77±2.60IU/L than the males with 47.91±4.84IU/L. At 6 months old the females had a highly significant ($p \leq 0.001$) value of 96.24 ± 1.80IU/L than the males with 49.00 ± 1.59IU/L. AST increased with increased age in both females and males (Table 3).
- **Glucose:** At 4 months old, the females had a higher highly significant ($p \leq 0.001$) value of 51.23±2.30mg/dl than the males with 44.21±2.05mg/dl. At 5 months old the females had a higher highly significant ($p \leq 0.001$) value of 67.60±3.67mg/dl than the males with 54.64±5.13mg/dl. At 6 months old the females had higher highly significant ($p \leq 0.001$) value of 83.00 + 0.70mg/dl than the males with 60.51 ± 0.87mg/dl. Glucose increased with increased age in both females and males (Table 3).

Discussion

The findings that PCV in the females were higher and highly significant ($p \leq 0.001$) than the males is not in agreement with the studies by Sniezesko (1960) who showed that sexually matured male brown trout (*Salmo gairdneri*) consistently had higher packed cell volume values than females and proposed it as means of gendering fish. The differences of this findings with that of Sniezesko (1960) may be due to species differences which are in agreement with Ranzani and Godinho (1991) that reported that differences PCV may be due to species differences, stage of maturation of gonads and also that blood parameters can change depending on the maturation of the gonads.

Hb in both females and males, increased in age and the females had a highly significant ($p \leq 0.001$) higher Hb than the males. The result suggests that with increase in age, there could be more stress in the females than the males with a physiological improvement in the oxygen transportation capacity of the blood to combat the reduced oxygen delivery capacity to tissues due to observed increases in PCV and the attendant stress. These findings are in agreement with studies by Wendelaar-Bonga, (1997) that increase in hemoglobin level is usually in fish subjected to stress; Mlay et al. (2007) that fish species, gender, treatment and worm infestation influenced hematological parameters and St.-Paul (1984) who suggested that the increase in Hb concentration could be a first indicator of an adaptational improvement in the oxygen transportation capacity of the blood.

RBC results reveals that the females had a higher highly significant means than the males at 4, 5 and 6 months old which increased with increased age in both the females and the males. Increase in RBC is an adjustment to maintain oxygen transfer to tissues. Also an increase in RBC increase is associated with the release of RBC from storage organ of the spleen through contraction and even cell division of circulating cells in fish subjected to low oxygen tension (Murad et al., 1993). As a result it appears that with increased age *C. gariepinus* experience low oxygen tension. However there is a limitation in increasing RBC count which is related to increase energy utilization to pump blood.

WBC in both the females and males had significant ($P \leq 0.05$) increase with age, which is evidence of increased mobilization with age of the immune system. However Gabriel et al. (2004) reported that sick *C. gariepinus* had higher WBC count than healthy *C. gariepinus* which may be mean that above certain level of WBC count means sick *C. gariepinus*. Lymphocyte result may mean that the females had a longer peak for lymphocyte production at 4 to 5 months unlike the males that was at 4 months before the significant ($P \leq 0.05$) decline. These may also suggest that the females based on its trait may have a better developed adaptive immune responds than the males at 4, 5 and 6 months of age.

Neutrophils results suggest that the production of neutrophils which is nonspecific cell of the immune system and highly motile and phagocytic and first to be found at the site of inflammation increased significantly ($P \leq 0.05$) earlier in the males at 5 months compared with the females at 6 months with a higher highly significant values. Monocytes results suggest that improvement in the production of monocyte in the females was delayed till 6 months while in the males, the production of monocyte was better, although with a decline at 5 months but with a subsequent improvement at 6 months. The lowest percentage was obtained in the females and males at 5 months of age. These may mean that *C. gariepinus* may have the lowest innate response at 5 months of age.

Erythrocyte Sedimentation Rate (ESR), results reveals that both the females and males had the highest level of ESR earlier in life at 4 month, thereafter experienced a decline with increase in age. The females lower values at 4 and 5 months

old but a higher highly significant (P≤0.001) at 6 months old may be indicative that at 6 months old after a period of non-significant difference at 5 months old, the female started a change from growth to reproductive process earlier than the male, a process that may be stressful.

The effective innate immune responses in fish is related to levels of serum protein, globulin and I$_g$m (Wiegertjes et al., 1996), while low level of plasma protein is associated with diseased fish (Wedemeyer et al., 1984). It thus appears that in the females' protein synthesis increases with increase in age than the males. Also these results may suggest that the females are healthier under normal circumstances than the males. However, according to Wedemeyer and Yasutaka (1977) elevated serum total protein may be attributed to the damage of kidney and gills as a result of exposure toxicants which is turn disturb osmoregulation.

Potassium and sodium at 4, 5 and 6 months old the females had a higher highly significant (p≤0.001) mean than the males of potassium and sodium. Also both potassium and sodium increased with increase age in both the females and males. Sodium is an extracellular electrolyte while potassium is intracellular electrolyte. Both are important for the proper functioning of cells, tissue and organ. Potassium is antagonist to sodium and the two ions are jointly balanced by chloride a negative ion. A significant decrease in plasma potassium may occur in response to severe hypoxia while in some species the levels of potassium were not affected as a result of stress. A decrease in levels of sodium is considered indices of secondary stress response. This decrease may be caused by loss of small ions through diffusion across the gill membrane which is associated with changes in gill due to an increase in brachial blood flow and permeability. Kori-siakpere (1996) documented a decrease in plasma electrolyte level as a indication of osmoregulatory impairment in experimental fish.

Creatinine, at 4, 5 and 6 months old, females had a higher highly significant (p≤0.001) means than the males also creatinine increased with increase in age. There is little to no tubular realsoption of creatinnine. Where there is kidney damage or deficiency, creatinine level rises. Creatinine levels are used to assess the functioning of the kidney. Elevated creatinine blood levels could be a sign of kidney problems or even failure of the kidney while low creatinine levels are indicative of decreased muscle mass.

ALT and AST, in the females were higher highly significant (p≤0.001) than in the males. Also, ALT and AST increased with increase age. Transaminases (ALT and AST) enzymes are frequency used to diagnose the sub-lethal damage to the different organs as well as liver (Rojik et al., 1983). Abbas (1994) recorded an increase in both aspartate and alanine transaminase of *0. niloticus* serum after exposure to 7, 14 and 21 mg Pb/l. Burtis and Ashwood (1996) reported that cell injury of organs leads to the release of tissue specific enzymes into the blood stream. Increased plasma activity of AST may be indicative of degenerative changes in myocardium, skeletal muscle, kidney and brain (Adeogun et al., 2012). Increased plasma ALT and AST activities are measurable parameters of cytolysis as aminotramferases play vital roles in carbohydrates-protein metabolism in fish (Eze, 1983). ALT and AST elevated levels may affect adversely amino acid metabolism (Kori Siakpere et al., 2010). It can be inferred that degenerative changes and adverse carbohydrates-protein metabolism in *C. gariepinus* increased with increase age in the female than the male.

Glucose at 4, 5 and 6 months old, the females had a higher highly significant means than the males. Glucose increased with increased age. The increase of glucose level indicates the presence of stressful stimuli which ellicitate rapid secretion of both glucorticoi and catecholarmmes from adrenal tissues (Mazeaud et al., 1977). Hyperglycemia was reported by Larsson et al. (1985) in case of freshwater species exposed to lead and cadmium. The result therefore suggests that with increase in age, there could be increase stress levels in the females than the males which could be physiological or adaptation/detrimental.

In summary, these results are in agreement with the findings that hematological parameter in fish may be influenced by intrinsic factors such as gender, reproductive stages, age, size and health (Joshi, 1982; Nespolo and Rosenmann, 2002) and thus recommended that the reference mean and range of *C. gariepinus* blood analysis based on gender and age (4 to 6 months table size production period) obtained may be reliable for diagnostic purposes during dry season.

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